Xylella fastidiosa gen. nov., sp. nov: Gram-Negative, Xylem-Limited, Fastidious Plant Bacteria Related to *Xanthomonas* spp.

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Twenty-five phenotypically and genotypically similar strains of a fastidious, xylem-limited bacterium were isolated from 10 plant disease sources including Pierce's disease of grapevines, phony disease of peach, periwinkle wilt, and leaf scorches of almond, plum, elm, sycamore, oak, and mulberry. The cells were single (occasionally filamentous), nonmotile, aflagellate straight rods (0.25 to 0.35 by 0.9 to 3.5 μm). They were gram negative, catalase positive, and oxidase negative, utilized hippurate, and produced gelatinase and often beta-lactamase but not beta-galactosidase, coagulase, lipase, amylase, phosphatase, indole, or H_2S. The bacteria were strict aerobes with optimum growth at 26 to 28°C and pH 6.5 to 6.9 and had doubling times of 0.45 to 1.98 days in periwinkle wilt broth. Monoclonal antibodies prepared against the Pierce's disease bacterium reacted with all strains. DNA composition was 51 to 53 mol% guanine plus cytosine, and strains were at least 85% related in DNA hybridization. Sequencing of 16S ribosomal ribonucleic acid related them to the xanthomonads. These bacteria form a distinct group, and the name *Xylella fastidiosa* is proposed, establishing a new genus with one species in the gamma subgroup of the eubacteria. Strain PCE-RR (ATCC 35879) is designated as the type strain.

Fastidious, gram-negative, xylem-limited procaryotes were first associated with diseases of plants in the Western hemisphere with the discovery of the bacterial etiology of Pierce's disease of grapevine (*Vitis vinifera* L.) by Davis et al. (7). Bacteria with similar morphology, habitat, and nutritional properties have been associated with phony disease of peach (*Prunus persica* [L.] Batsch), wilt of Madagascar periwinkle (*Catharanthus roseus* [L.] G. Don), stunting of ragweed (*Ambrosia artemisiifolia* L.), and leaf scorches of almond (*Prunus amygdalus* L.), Japanese plum (*Prunus saliciana* Lindl.), elm (*Ulmus americana* L.), sycamore (*Platanus occidentalis* L.), oak (*Quercus rubra* L.), mulberry (*Morus rubra* L.), and maple (*Acer rubrum* L.) (13, 16, 26, 27, 33, 36, 37, 41, 44, 45; J. Sherald, unpublished data). Reports from southeast Asia and Australia associate small, fastidious, xylem-limited bacteria with Sumatra disease of cloves (1), and crinkle leaf disease of kenaf (*Hibiscus cannabinus* L.) (G. M. Behncken, Department of Primary Industries, Brisbane, Australia, personal communication). Bacteria associated with these diseases are gram negative, catalase positive, obligately aerobic, similar in morphology and grow slowly on specialized media with doubling times of 9 h to 2.3 days (9; J. Wells, unpublished results). They are small slender rods (0.25 to 0.35 by 0.9 to 3.5 μm), with filamentous forms common on artificial media (6, 45). Furrowed or rippled cell walls resembling those of the families *Rickettsiaceae* and *Legionellaceae* (4, 9, 28) are a typical ultrastructural feature; hence, there are occasional references to rickettsialike bacteria. Cellular fatty acid composition of strains from different plant hosts is similar, with a high percentage of saturated, odd-carbon straight chains (6 to 18%) and no cyclopropane acids (43). Independent DNA analyses agree on a value of 50.1 to 54.0 mol% guanine plus cytosine (G+C) and a genome size of 1.4×10^9 daltons (9, 15).

There are no published data on the phylogeny of the fastidious bacteria or on their relatedness to the classically recognized genera of gram-negative bacteria, such as *Pseudomonas* (group 1), *Xanthomonas*, *Escherichia*, and *Nitrobacter*. These are now included in the gamma subgroup of the eubacteria, described extensively in the literature as the gamma subdivision of the purple bacteria (11, 48). This report examines properties of 25 strains of the xylem-limited bacteria isolated from 10 different disease sources, including 4 strains of the Pierce's disease bacterium. Presented are results of physiological and biochemical tests intended to help clarify two taxonomic issues: the degree of internal homogeneity among strains of these bacteria and the phylogenetic relationship of the group to known phytobacteria.

MATERIALS AND METHODS

Bacterial strains and media. Twenty-five strains derived from primary isolations or obtained from contributors were cloned three times by propagation of single colonies on buffered cysteine-yeast extract (BCYE) agar medium (45). Strains of the Pierce's disease bacterium, PCE-VT (ATCC 33107), PCE-GG (ATCC 35877), PCE-RR (ATCC 35879), and PCE-FG (ATCC 35881), and strains from almond leaf scorch, ALS (PDDCC 8739) and ALS-BC (ATCC 35870), were isolated on JD-3 agar (7) from infected leaf petioles. Strains from plum leaf scorch, PLM-G83 (ATCC 35871), PLM-84 (PDDCC 8743), and PLM-85, and from phony disease of peach, PCH-83 (PDDCC 8744) and PCH-84, were isolated on BCYE agar from infected twigs and roots, respectively (45). Periwinkle strains PWT-17 (PDDCC 8738), PWT-22 (ATCC 35878), and PWT-100 (ATCC 35880) were originally isolated on periwinkle wilt (PW) medium, BCYE, and sycamore scorch medium by Davis et al. from wilt-

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infected stems (8). Ragweed strains RGW-1265 and RGW-R (ATCC 35876) were isolated from infected stems by Timmer et al. on PW medium (41). Strains from elm, ELM-ES6 (ATCC 35873), ELM-2 (ATCC 35872), and ELM-6, mulberry, MUL-1 (ATCC 35868), MUL-3 (ATCC 35869), and MUL-V, and oak, OAK (ATCC 35874) and OAK-BC, were isolated by Kostka et al. from chips of infected twigs in PW broth medium (16; S. Kostka, personal communications). The sycamore strain, SYC-1 (PDDCC 8740), was isolated by Sherald et al. on sycamore scorch medium (36). All strains were maintained on BCYE agar medium and in PW broth cultures (with continuous rotary agitation) at 27°C and subcultured every 30 days. Most strains were stored in freeze-dried condition and deposited at the American Type Culture Collection or Plant Disease Division Culture Collection or both. After 12 to 18 months of subculturing, new stock cultures were isolated from infected host plants or revived from freeze-dried stocks.

General methods and biochemical tests. Biochemical tests were done within 6 months of isolation or acquisition of the strains. All reagents were obtained from Sigma Chemical Co., St. Louis, Mo., unless otherwise noted. Gram staining and acid-fast staining were done by the methods of Hucker and Ziehl-Neelsen, respectively (10). Cell and colony morphology were examined by phase-contrast microscopy of unstained preparations, by transmission electron microscopy of preparations negatively stained with uranyl acetate and lead citrate (21), and by scanning electron microscopy by methods previously described (33). Catalase production was tested on a microscope slide by mixing bacteria from 10- to 14-day-old BCYE cultures with 3% hydrogen peroxide. Oxidase was tested with Taxo N oxidase discs (BBL Microbiology Systems, Cockeysville, Md.) which were moistened and placed on 14-day-old colonies growing on BCYE agar. No color change within 24 h indicated negative results. Urease was tested with cultures growing in PW broth (with phenol red) amended with 2% urea (38). Controls were grown in medium lacking urea. An alkaline shift (red-violet color) greater than that of the controls was considered positive. The optimum temperature, atmosphere, and pH for growth on BCYE agar were determined by methods previously described (45). Optima were also determined in flasks containing 20 ml of PW broth inoculated with 1 ml of a 7- to 10-day-old culture containing 10^7 to 10^8 cells per ml and incubated with agitation for 10 to 14 days at 27°C or within a range of 15 to 40°C. Growth response was determined by cell counts or by turbidity at 640 nm. Controlled atmospheres were tested with flasks fitted with gas inlets and connected to lines through which the following premixed, humidified gases flowed (at 27°C): 1 or 5% O_2 (balance, N_2), 2.5 or 5% CO_2 with 21% O_2 (balance, N_2), or air. Medium pH from 5.8 to 7.5 was tested in 0.1-U increments. Tolerance to NaCl was tested in PW medium amended with 0.5, 1, 2, 3, and 5% NaCl. The effects of supplementary carbon sources on growth were tested in PW medium with equimolar concentrations of other carbon and nitrogen sources in place of L-glutamine. Carbohydrates and amino acids (L isomers) tested at 0.03 M were alanine, arginine, asparagine, cysteine, glutamic acid, methionine, serine, L-arabinose, D-galactose, D-glucose, fructose, D-mannose, ribose, trehalose, D-xylose, choline, citrate, esculin, α-ketoglutarate, malonate, succinate, and urea. The data are considered approximate because of undetermined levels of carbohydrates in peptones.

Tests for extracellular enzymes. Protease and amylase activity was measured by culturing strains on PW agar supplemented with 1% acid hydrolysate of casein, *N,N*-dimethylated casein (20), or 1% soluble starch. Lipase was detected on PW agar supplemented with 10% fresh hen-egg yolk or 1% Tween 80 (38, 40). Plates were heavily streaked and incubated for 10 days at 27°C. Zones of hydrolysis indicated positive responses. Coagulase was tested with commercial plasma (Difco Laboratories, Detroit, Mich.) by the tube method, and gelatinase was tested with Kohn gelatin-charcoal discs added to 7-day-old cultures in PW broth and incubated for 7 days (23). The positive reference culture for protease, starch hydrolysis, and coagulase was *Erwinia carotovora* ATCC 15713; *Xanthomonas campestris* ATCC 11551 was used for lipase. Beta-lactamase was detected with penicillin-starch paper strips (30). Phosphatase was tested on PW agar supplemented with 0.5% phenolphthalein diphosphate (40) and by assay of supernatants of 21-day-old PW broth cultures with *p*-nitrophenyl phosphate (39). Deoxyribonuclease and ribonuclease (RNase) were tested on PW agar amended with 0.2% highly polymerized deoxyribonucleic acid (DNA) (type I from calf thymus) or 0.2% ribonucleic acid (RNA) (type II-C from *Torula* yeast cells). Zones of hydrolysis were measured after 7 days of incubation (40).

Serology. Serological characteristics were determined by indirect enzyme-linked immunosorbent assay (ELISA) with an avidin-biotin-peroxidase complex (14) and an enzyme immunoassay automatic analyzer system (PR-50; Gilford Instrument Laboratories, Inc., Oberlin, Ohio). Bacteria were grown for 30 days on BCYE agar. Antigens for coating microtiter plates were prepared by diluting sonicated suspensions in 0.05 M carbonate buffer (pH 9.6) to 10 to 20 μg of protein per well (0.2 to 0.4 mg). Antibodies were prepared as previously described from sera of New Zealand White rabbits immunized with the Pierce's disease bacterium (33). Monoclonal antibodies were secreted from hybridoma cells produced by fusion of NS-1 myeloma cells and splenic cells of a BALB/c mouse immunized with strain PLM-G83 of the plum leaf scorch bacterium by methods described by Lin and Chen (19). Screening was against strains PCE-RR, PLM-G83, and PWT-100. Ascitic fluid antibody was obtained from Swiss Webster mice immunized against strain PLM-G83. Fluid was induced by an intraperitoneal injection of 10^6 Ehrlich-Lettre ascites carcinoma cells 24 days after immunization, followed by a booster injection of antigen on day 30. Ascitic fluid was collected on day 33 and clarified by centrifugation. Immunoglobulins were purified by precipitation with 50% saturated ammonium sulfate and by dialysis (35). Dilutions of rabbit antibody for ELISA reactions were done as described previously (29). Ascitic fluid immunoglobulins were diluted 1:1,200, 1:2,400, and 1:4,800. Monoclonal antibodies were standardized at 1 to 5 μg of protein per well (20 to 100 μg/ml). The ELISA results are expressed as average optical density readings at 490 nm from three separate determinations. Values greater than 0.1 were considered positive since nonspecific background readings for normal serum (negative control) were less than 0.1.

Fatty acid analysis. Total cellular fatty acid composition was determined by gas-liquid chromatography of methyl esters with a Varian Model 3700 Gas Chromatograph (Varian Associates, Sunnyvale, Calif.) equipped with a fused silica capillary column (15 m by 0.25 mm). Suspensions of washed bacterial cells grown on BCYE agar at 27°C were saponified and then methylated by the method of Maas et al. (22). Chromatography conditions, identification and confirmation of individual fatty acids, and data integration were as previously described (43).

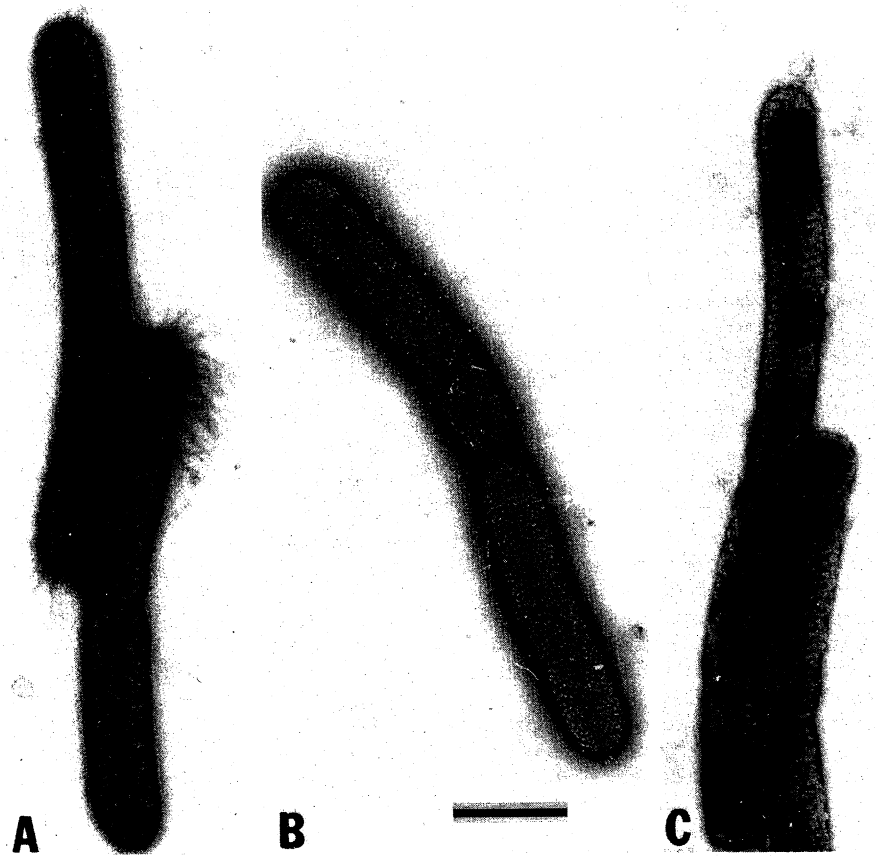

FIG. 1. Negatively stained preparations of gram-negative, xylem-limited, fastidious bacteria from grapevines affected with Pierce's disease (A), ragweed affected with stunt disease (B), and peach affected with phony disease (C). The rods shown are 0.25 to 0.27 by 1.2 to 2.4 μm, have furrowed or convoluted cell walls, and appear to have undergone binary fission. The fimbriae in some strains are peritrichous but are more evident or denser on polar regions. Bar = 0.5 μm. Magnification, ×21,000.

DNA analyses. DNA was isolated from cells grown in PW broth at 27°C for 21 days by the method of Mandel et al. (24). The G+C content of selected strains was determined by buoyant density centrifugation in cesium chloride (CsCl) and spectrophotometrically by thermal denaturation (25). Samples were centrifuged in CsCl at 200,000 × *g* for 23 h at 25°C in duplicate. References for density were DNAs from phage 2C (density, 1.742 g/cm^3) from *Escherichia coli* K-12 (density, 1.710 g/cm^3). Preparation of DNA labeled in vitro with $^{32}PO_4$ and determination of DNA relatedness by the hydroxyapatite method have been described (2). Tritium-labeled gene probes specific for the families *Enterobacteriaceae* and *Legionellaceae* (provided by D. E. Kohne, Gen-Probe Inc., San Diego, Calif.) were tested by the hydroxyapatite method at 60 and 65°C, respectively.

RNA analysis. The 16S ribosomal RNA (rRNA) was sequenced directly from unfractionated cellular RNA from early-log-phase cells grown in PW broth for 3 to 7 days. Sequencing was done by the dideoxynucleotide chain termination method using reverse transcriptase and gene-specific oligonucleotide primers (17, 32). The sequence for strain PWT-100 was determined from the 5′ end to position 1489 (*E. coli* numbers) (5, 46). Positions 148 to 320 were determined for strain PLM-G83. A similarity matrix was constructed with other eubacterial sequences by using a data base that included approximately 400 partial (oligonucleotide) 16S rRNA sequences from bacteria (11, 48; C. R. Woese, personal communication). Sequences from the following bacteria were selected, each representative of a major phylogenetic group: *Agrobacterium tumefaciens* (alpha subdivision of purple bacteria) (42, 47), *Pseudomonas testosteroni* (beta subdivision of purple bacteria) (31, 49), *Chromatium vinosum* and *Legionella pneumophila* (gamma subdivision of purple bacteria) (48; C. R. Woese, personal communication), *Desulfovibrio desulfuricans* (delta subdivision of purple bacteria) (48; C. R. Woese, personal communication), and *Bacillus subtilis* (gram positive) (12). Sequences were decomposed into RNase T1 oligonucleotides, stretches of bases that contain no guanosine but end in a single G. These were compared by signature analysis with existing T1 oligonucleotide catalogs available for various bacteria including *Xanthomonas maltophilia* and *X. campestris* (47–49).

RESULTS

Morphology and cultural characteristics. All strains of the xylem-limited bacteria grew on BCYE or PW medium but not on standard bacteriological media such as Trypticase soy agar (BBL), nutrient agar, or King B medium. Colonies were discrete, opalescent, and circular, measuring up to 0.6 mm in diameter after 10 days at 27°C and up to 1.5 mm after 30 days. Two colony types, smooth opalescent and rough opalescent, were observed with all strains. When viewed by reflected light on the dark charcoal background of BCYE agar, the margins of rough colonies were tinged with green or

red. The cellular dimensions of all strains were 0.25 to 0.35 by 0.9 to 3.5 μm. The main morphological form of the smooth colonies, when freshly isolated on BCYE agar, was tightly intertwined filamentous strands. Rough colonies were composed of single rod-shaped cells. Liquid cultures were generally composed of dispersed, single cells, but some strains formed aggregations. Long filaments were also seen in cultures in most strains growing in PW broth supplemented with 0.05 M mannose. In PW broth, log-phase growth generally ended after 7 to 10 days. Maximum concentrations, ranging from 2.0×10^7 to 6.0×10^8 cells/ml occurred after 7 to 14 additional days of stationary-phase growth. Doubling times (T_ds) were 0.45 to 1.98 days, with an average of 0.91 days. In BCYE broth T_ds were 0.95 to 2.41 days, with an average of 1.65 days. Optimum growth temperatures were 26 to 28°C, and the optimum pH range was 6.5 to 6.9. Final culture pHs in PW and BCYE media ranged from 7.2 to 7.8.

Negatively stained preparations of all strains examined by transmission and scanning electron microscopy revealed rod-shaped cells with rounded ends or with one tapered end and showing numerous irregular ridges or folds on cell wall surfaces (Fig. 1). Fimbrialike fibrils were peritrichous and particularly conspicuous at the poles, but there was no evidence of flagella. Cross sections showed spherical to ovoid cells with wall profiles of the gram-negative type: an outer membrane, an electron-dense layer, and an inner, smooth cytoplasmic membrane. Ribosomes, vesicles, mesosomes, filaments, and DNA-like granules were present in the cytoplasm. Cells were seen in the process of binary fission.

Physiological and biochemical tests. Strains were gram negative, oxidase negative, catalase positive, hydrolyzed gelatin, utilized hippurate, and did not produce indole, H_2S, beta-galactosidase, lipase, amylase, coagulase, or phosphatase. Most strains produced beta-lactamase. They were strictly aerobic and were inhibited by 2.5% CO_2. The growth of all strains in PW broth was stimulated when D-galactose, L-glutamine, L-methionine, mannose, or trehalose was substituted for L-glutamine. Over 90% of the strains were stimulated by D-glucose and L-arginine, and 45 to 50% were stimulated by L-cysteine. Substitutions of L-arabinose, L-alanine, L-asparagine, L-choline, citrate, esculin, fructose, L-glutamic acid, α-ketoglutarate, malonate, L-serine, succinate, urea, and D-xylose generally inhibited growth. NaCl was completely inhibitory to growth in PW medium at 3 M. At 0.5 to 1.0 M NaCl, final cell concentrations were reduced by approximately 50%.

Serology. Antibodies prepared in rabbits against the Pierce's disease and plum leaf scorch bacteria reacted with all tested strains of the fastidious plant bacteria but not with other bacteria (Table 1). Ascitic fluid antibody to strain PCE-RR also reacted with all strains but produced cross-reactions with several gram-negative phytopathogens including *X. campestris*. Monoclonal antibodies produced from two different fusions, reacted only with the fastidious bacteria.

Fatty acid composition. The composition of total cellular fatty acids of all strains of the xylem-limited bacteria was similar and was not affected by the physiological age of the cells. The major components (and percentages of the total) were $C_{16:0}$ (30%), $C_{16:1}$ (26.7%), $C_{17:0}$ (11.6%), and $C_{15:0}$ (8%) fatty acids (Table 2). This profile was unlike that of any other genera tested and unlike any analysis reported (18). The percentage of saturated, odd-numbered carbon straight chains was noticeably higher (18.2%) than in other gram-negative phytopathogens, and cyclopropane acids were entirely absent (Table 3).

TABLE 1. Serologic reactions in indirect ELISA of antibodies prepared against Pierce's disease bacterium, strain PCE-RR

Antigen	ELISA OD reading at 490 nm		
	Immunized rabbit antiserum[a]	Mouse ascitic fluid antibody[b]	Monoclonal antibody[c]
Xylem-limited bacteria			
PCE-RR	++	0.322 ± 0.055	0.197 ± 0.028
PCE-GG	++	0.203 ± 0.023	0.206 ± 0.035
ALS	++	0.327 ± 0.045	0.236 ± 0.001
PLM-G83	+	0.251 ± 0.037	0.307 ± 0.032
PLM-84	+	0.238 ± 0.055	0.286 ± 0.034
PCH-84	+	ND	0.336 ± 0.017
PWT-22	+	0.109 ± 0.031	0.278 ± 0.032
PWT-100	+	0.124 ± 0.019	0.375 ± 0.022
RGW	ND	0.115 ± 0.054	0.228 ± 0.067
ELM-1	++	0.303 ± 0.069	0.365 ± 0.058
ELM-2	++	0.329 ± 0.028	0.571 ± 0.056
OAK	ND	0.159 ± 0.058	0.394 ± 0.029
SYC	ND	0.222 ± 0.035	0.213 ± 0.019
MUL-1	ND	0.283 ± 0.046	0.353 ± 0.036
Xanthomonas campestris ATCC 11551	−	0.134 ± 0.004	0.012 ± 0.016
Pseudomonas syringae ATCC 11965	−	0.054 ± 0.018	0.068 ± 0.008
Erwinia carotovora ATCC 15713	−	0.120 ± 0.016	0.022 ± 0.005
Erwinia amylovora NCPPB-595	−	0.076 ± 0.029	0.018 ± 0.011
Corn stunt spiroplasma (ATCC 20951)	ND	0.038 ± 0.005	0.018 ± 0.002
Rickettsia rickettsii[d]	ND	0.081 ± 0.022	0.023 ± 0.006
Rickettsia typhi[d]	ND	0.051 ± 0.007	0.030 ± 0.006
Legionella micdadei[d]	ND	0.042 ± 0.012	0.005 ± 0.003
Legionella pneumophila[d]	ND	0.087 ± 0.014	0.008 ± 0.002

[a] Serum antibody was diluted 1:1,500; antigen was standardized at 20 μg of protein per well. ELISA reactions were rated visually: ++, strong; +, weak; −, negative. ND, Not determined.

[b] Ascitic fluid was diluted 1:2,400; the antigen concentration was 10 μg of protein per well. Data are averages of three replications ± standard error.

[c] The antibody concentration was 5 μg of protein per well; the antigen concentration was 10 μg of protein per well. Data are averages of three replications ± standard error of monoclone EF4.

[d] Lyophilized antigen was obtained from Centers for Disease Control, Atlanta, Ga.

DNA composition and relatedness. The buoyant density of the DNA was 1.710 g/cm^3 for strains PCE-VT, PCE-RR, PLM-G83 and PCH-83, and 1.712 g/cm^3 for strains RGW-1265 and ELM-ES6, which equated to 52.0 and 53.1 mol% G+C, respectively. With the thermal denaturation method, the mol% G+C of strains PCE-RR, PLM-G83, and PWT-100 was 52.4, 51.0, and 51.4, respectively. Total DNA from strain PCE-RR, labeled in vitro with $^{32}PO_4$, was highly related to unlabeled DNAs from the two other strains of fastidious bacteria tested (greater or equal to 85% relatedness with 2% divergence within related sequences) but was essentially unrelated to DNAs of the other bacteria tested, including DNAs representing 18 *Legionella* species (3) (Table 4). Similarly, the three xylem-limited strains showed very low reactivity (6% or less) with family-specific gene probes for the *Enterobacteriaceae* and *Legionellaceae* (Table 4).

RNA sequence analysis. The comparable regions of the 16S rRNA from PWT-100 and PLM-G83 were identical in sequence, as would be expected from DNA-DNA hybridization data. Values for similarity of the sequences to those of

TABLE 2. Distribution of fatty acids in strains of the fastidious, xylem-limited bacteria

Fatty acid[a]	% in bacteria from[b]:					Avg %[c]
	Grape	Plum	Periwinkle	Mulberry	Elm	
8:0	0.3	2.1	0.3	1.0	0.4	0.82 ± 0.35
10:0	1.1	1.1	1.4	1.4	1.1	1.22 ± 0.07
2OH-10:0	1.5	0.8	1.3	0.8	2.4	1.36 ± 0.29
3OH-12:0	0.4	0.2	0.5	0.1	2.2	0.68 ± 0.38
anteiso-15:0	0.4	0.5	0.5	1.5	0.8	0.74 ± 0.20
15:0	7.2	5.2	4.8	17.4	5.2	7.96 ± 2.40
16:1	33.3	22.5	29.9	25.2	22.8	26.74 ± 2.11
16:0	37.8	25.4	30.8	27.8	28.5	30.06 ± 2.12
iso-17:0	1.7	4.8	4.6	3.5	5.3	3.98 ± 0.64
anteiso-17:0	1.3	5.5	3.0	3.7	4.6	3.62 ± 0.72
17:0	4.7	18.2	11.3	10.0	13.9	11.62 ± 2.23
18:2	1.8	5.2	5.4	1.5	4.5	3.68 ± 0.84
18:1	2.5	2.0	1.0	0.1	2.9	1.70 ± 0.51
Unknowns	0.7	0.7	0.7	0.4	1.2	0.75 ± 0.13

[a] Number of carbon atoms:number of double bonds. Fatty acids averaging more than 0.75% of total cellular fatty acids are shown.
[b] Average of three determinations per strain, with three strains per source.
[c] Average for strains ± standard error.

other bacteria place the fastidious plant pathogens within the gamma subgroup of the eubacteria (i.e., the gamma subdivision of the purple bacteria), represented in these tests by *C. vinosum* and *L. pneumophila*, but which includes *Xanthomonas* spp. (Table 5). Signature analysis of the T_1 oligonucleotides identified the PWT-100 sequences as closely and specifically related to sequences within a group including *X. campestris*, *X. maltophila* (Table 6), and *Lysobacter enzymogenes* (48). The oligonucleotide sequences AAACCCUACUCAG and AACAUCCG are unique, among all eubacteria, to these four species. The oligonucleotides AAUACAUCG and AACAUCCG are unique to the xylem-limited bacteria and one or both of the xanthomonads. A similarity coefficient of 0.39 excluded any close specific phylogenetic relationship between the fastidious bacteria and *L. pneumophila*. (Table 6).

DISCUSSION

The gram-negative, xylem-limited fastidious bacteria studied constitute a homogenous group. Comments are necessary, however, regarding strain variability, gaps in our knowledge, and phylogeny.

Although all strains were nutritionally fastidious (narrowly defined as requiring a specialized medium such as PW or BCYE for primary isolation and growth), significant differences existed in growth rates. Strains with high growth rates were isolated from grape (Pierce's disease), almond, elm, and mulberry. T_ds in PW broth for these strains ranged from 0.5 to 1.6 days. Davis et al. reported a T_d of 9 h for the Pierce's disease bacterium (9). The strains from peach and plum were the most difficult to grow, with T_ds as long as 2.0 days. Determination of the significance, if any, of strain variability and an understanding of the nutritional requirements of these bacteria will have to await the development of a chemically defined medium.

Serological data support the hypothesis that the fastidious xylem-limited bacteria are a homogenous group. The weak heterologous reactions with polyclonal rabbit antiserum (33) and some strain selectivity with monoclonal antibodies (J. Wells, unpublished data) do not suggest serotypes. Epitopes exclusive to the fastidious bacteria can be inferred from the absence of cross-reactions of monoclonal antibodies with other bacterial groups. The cross-reactions of ascitic fluid antibody with *E. carotovora* and *X. campestris* suggest that there are epitopes shared with other bacteria, an antigenic phenomenon not uncommon among plant pathogenic bacteria (34).

Fatty acid analysis is a supportive taxonomic tool to distinguish between genera and some species of bacteria (18). The profiles for all strains of the fastidious bacteria were similar and unlike those of any other gram-negative phytopathogens. In a previous study of the fatty acids of fastidious bacteria (43), conducted with a gas-liquid chromatograph equipped with packed columns, the percentage of branched-chain fatty acids averaged 1.2% of the total, significantly lower than the 8.5% found in the present study. This discrepancy is due to components earlier categorized as unknown unsaturated acids (based on confirmatory tests) and now identified as branched-chain fatty acids with unsaturated moieties.

The results of DNA hybridization studies leave no doubt that xylem-limited fastidious bacteria are a single species. The most significant problem in fully characterizing them, however, is their phylogenetic relationship to other bacteria. Earlier speculations concerning an association with the order *Rickettsiales* were based on morphological resemblances, further strengthened by their nutritional fastidiousness. They were also first isolated with modifications of media developed for *Rochalimaea* and *Legionella* spp. (9, 45). The resemblances, however, are superficial. Reactions of DNAs from fastidious bacteria with gene probes prepared from *L. pneumophila* and *E. coli* were 5 to 10 times lower than those of DNAs from the *Enterobacteriaceae* and *Legionellaceae*. These data indicate that the xylem-limited fastidious bacteria do not belong to either family.

Comparison of 16S rRNA sequences by similarity and by

TABLE 3. Fatty acid profiles of the fastidious, xylem-limited bacteria and selected plant pathogens

Fatty acid class	% (mean ± SE) of total fatty acids in[a]:			
	Fastidious bacteria	*Pseudomonas syringae*	*Xanthomonas campestris*	*Erwinia amylovora*
Saturated, even-carbon straight chains	33.5 ± 2.2	25.7 ± 1.0	11.0 ± 1.0	42.1 ± 2.0
Saturated, odd-carbon straight chains	18.2 ± 2.4	0.6 ± 0.1	1.8 ± 0.4	5.1 ± 0.9
Unsaturated acids	34.8 ± 1.7	64.2 ± 0.9	14.1 ± 1.5	34.8 ± 1.5
Hydroxy acids	3.1 ± 0.5	7.3 ± 0.6	1.7 ± 0.5	6.4 ± 0.8
Branched-chain acids	8.5 ± 1.2	0.3 ± 0.1	65.4 ± 4.7	0.7 ± 0.3
Cyclic acids	0	0.2 ± 0.2	0.2 ± 0.1	4.3 ± 0.5
Unidentified components	1.7 ± 0.5	1.7 ± 0.7	5.3 ± 0.4	5.4 ± 0.5
Ratio of saturated/unsaturated acids	1.51 ± 0.17	0.41 ± 0.01	0.96 ± 0.10	1.40 ± 0.21
Ratio of even-odd-carbon saturated straight chains	1.62 ± 0.84	52.57 ± 19.11	6.85 ± 1.03	18.96 ± 0.93

[a] Averages for 23 strains of fastidious bacteria, 12 strains of *P. syringae*, 10 strains of *X. campestris*, and 12 strains of *E. amylovora*.

TABLE 4. Relatedness of DNA from strains of the fastidious, xylem-limited bacteria to total DNA of other bacteria and to family-specific gene probes

Source of unlabeled DNA	Relatedness of[a]:				
	Labeled DNA from strain PCE-RR			Labeled gene probe to:	
	RBR, 60°C	%D	RBR, 75°C	*Legionellaceae* (RBR, 65°C)	*Enterobacteriaceae* (RBR, 60°C)
Xylem-limited bacteria					
PCE-RR	100	0.0	100	2	6
PLM-G83	99	2.0	98	2	6
PWT-100	85	2.0	87	3	5
Legionella spp.	2[b]			100	10
Legionella pneumophila Philadelphia 1	2			34	9
Legionella bozemanii WIGA	2			32	5
Legionella dumoffii NY-23	2			4	100
Escherichia coli K-12				1	45
Klebsiella oxytoca ATCC 13182					25
Proteus penneri CDC 1808-73					

[a] $^{32}PO_4$-labeled DNA was reacted with unlabeled DNAs. The control value (2.4%) was subtracted before normalization. RBR (relative binding ratio) = (percentage of DNA bound to hydroxyapatite in heterologous reaction/percentage of DNA bound in homologous reaction) × 100, before normalization. Homologous DNA from strain PCE-RR reassociated to an average of 70% at 60°C and 57% at 75°C. Percent divergence (%D) was based on the assumption that each 1% decrease in the thermal stability of the heterologous DNA duplex compared with that of the homologous duplex was due to 1% unpaired bases.

[b] Average for 18 species.

signature analysis provided evidence relating the fastidious bacteria to the xanthomonads. The association of these bacteria with the gamma subgroup of the eubacteria excludes any phylogenetic relationship to the rickettsiae.

It appears that this group of bacteria constitutes a new species and that they are phenotypically and genotypically different enough from their closest relatives, the xanthomonads, to merit separate genus status. Whether the new genus should be in a separate family cannot be decided without resolution of the complex taxonomic problems in the family *Pseudomonadaceae*, which contains the genus *Xanthomonas*. We therefore propose the name *Xylella fastidiosa* gen. nov., sp. nov. for the xylem-limited bacteria.

Description of *Xylella* gen. nov. *Xylella* (xy. le. la. Gr. n. *xylon* wood; M. L. dim. ending *ella*). Predominantly single, straight rods, 0.25 to 0.35 by 0.9 to 3.5 μm, with long, filamentous strands under some cultural conditions. Colonies are of two types: convex to pulvinate smooth opalescent with entire margins and umbonate rough with finely undulated margins. They are gram-negative, nonmotile, aflagellate, oxidase negative, catalase positive, strictly aerobic, nonfermentative, nonhalophilic and nonpigmented bacteria. They are nutritionally fastidious, requiring a specialized medium such as BCYE containing charcoal or glutamine-peptone medium (PW) containing serum albumin. Optimal temperature for growth is 26 to 28°C, and optimum pH is 6.5 to 6.9. Their habitat is the xylem of plant tissue. The G+C content of the DNA is 51.0 to 52.4 mol% (determined by thermal denaturation) or 52.0 to 53.1 mol% (determined by buoyant density). The type species is *Xylella fastidiosa*.

Description of *Xylella fastidiosa* sp. nov. *Xylella fastidiosa* (fas. tid. i. o'sa. N. L. m. adj. *fastidiosus*, highly critical; referring to the nutritional fastidiousness of the organism, particularly on primary isolation). Its characteristics are those of the genus, as listed above. It hydrolyzes gelatin and utilizes hippurate, and most strains produce beta-lactamase. It does not ferment D-glucose and is negative in tests for indole, H_2S, beta-galactosidase, lipase, amylase, coagulase, and phosphatase. It has been isolated as a phytopathogen from tissues of a number of host plants. The type strain is PCE-RR (=ATCC 35879).

Description of type strain. Strain ATCC 35879^T has all the characteristics given above for the genus and species. The G+C content of the DNA is 52.4 mol% (thermal denaturation) or 53.1 mol% (buoyant density). It was isolated from grapevine with Pierce's disease.

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TABLE 5. Similarity of 16S rRNA sequences from the fastidious, xylem-limited bacteria to other eubacterial sequences

Bacterium	% Similarity to:						
	Xylem-limited bacteria[a]	*A. tumefaciens* (alpha)[b]	*P. testosteroni* (beta)[b]	*C. vinosum* (gamma)[b]	*L. pneumophila* (gamma)[b]	*D. desulfuricans* (delta)[b]	*B. subtilis* (gram positive)
Xylem-limited							
Agrobacterium tumefaciens	81.6						
Pseudomonas testosteroni	83.0	78.8					
Chromatium vinosum	85.7	83.1	84.5				
Legionella pneumophila	85.1	81.2	82.2	87.4			
Desulfovibrio desulfuricans	80.3	80.6	78.4	81.5	79.8		
Bacillus subtilis	79.1	79.2	77.5	81.1	78.3	80.6	

[a] The sequence for strain PWT-100 was determined from the 5′ end to position 1489 (*E. coli* numbers) and for strains PLM-G83 and PCE-FG from positions 148 to 320.

[b] Shown in parenthesis is the subdivision of the purple bacteria (eubacteria) to which the organism belongs.

TABLE 6. Similarity coefficients between 16S rRNA sequences of selected bacteria and the xylem-limited fastidious bacteria

Bacterium	Similarity coefficient for:				
	Xylem-limited bacteria	*X. maltophilia*[a]	*X. campestris*[a]	*C. vinosum*[a]	*L. pneumophila*[a]
Xylem-limited *Xanthomonas maltophilia*[b]	0.65[c]				
Xanthomonas campestris	0.65	0.85			
Chromatium vinosum	0.39	0.44	0.45		
Legionella pneumophila	0.39	0.39	0.40	0.45	

[a] Member of gamma subdivision of the purple bacteria.
[b] *Xanthomonas* phylogeny originally described in Woese et al. (48).
[c] Similarity coefficient based on matrices constructed with approximately 400 partial 16S rRNA sequences (11).

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